



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07C 259/06, A61K 31/185	A1	(11) International Publication Number: WO 97/43250 (43) International Publication Date: 20 November 1997 (20.11.97)
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(21) International Application Number: PCT/EP97/02500
(22) International Filing Date: 7 May 1997 (07.05.97)
(30) Priority Data:
9609795.1 10 May 1996 (10.05.96) GB

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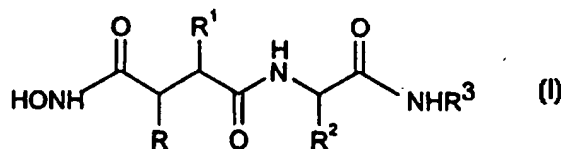
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(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published

With international search report.

(54) Title: HYDROXAMIC ACID BASED INHIBITORS OF THE FORMATION OF CD23 AND TNF



(57) Abstract

Compounds of formula (I) wherein: R is (C₂₋₆)alkenylthiomethyl or (C₂₋₆)alkynylthiomethyl; R¹ is alkyl or alkenyl; R² is alkyl, alkenyl, aryl, cycloalkyl or cycloalkenyl; and R³ is hydrogen, alkyl, alkenyl, alkynyl or aryl; are useful in the treatment of disorders mediated by s-CD23.

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HYDROXAMIC ACID BASED INHIBITORS OF THE FORMATION OF CD 23 AND TNF

This invention relates to novel inhibitors of the formation of soluble human CD23 and their use in the treatment of conditions associated with excess production of soluble CD23 (s-
5 CD23) such as autoimmune disease and allergy. The compounds of the invention are also inhibitors of the release of tumour necrosis factor (TNF).

CD23 (the low affinity IgE receptor FcεRII, Blast 2), is a 45 kDa type II integral protein expressed on the surface of a variety of mature cells, including B and T lymphocytes, macrophages, natural killer cells, Langerhans cells, monocytes and platelets (Delespesse *et al*,
10 *Adv Immunol*, 49 [1991] 149-191). There is also a CD23-like molecule on eosinophils (Grangette *et al*, *J Immunol*, 143 [1989] 3580-3588). CD23 has been implicated in the regulation of the immune response (Delespesse *et al*, *Immunol Rev*, 125 [1992] 77-97). Human CD23 exists as two differentially regulated isoforms, a and b, which differ only in the amino acids at the intracellular N-terminus (Yokota *et al*, *Cell*, 55 [1988] 611-618). In man
15 the constitutive a isoform is found only on B-lymphocytes, whereas type b, inducible by IL4, is found on all cells capable of expressing CD23.

Intact, cell bound CD23 (i-CD23) is known to undergo cleavage from the cell surface leading to the formation of a number of well-defined soluble fragments (s-CD23), which are produced as a result of a complex sequence of proteolytic events, the mechanism of which is
20 still poorly understood (Bourget *et al J Biol Chem*, 269 [1994] 6927-6930). Although not yet proven, it is postulated that the major soluble fragments (Mr 37, 33, 29 and 25 kDa) of these proteolytic events, all of which retain the C-terminal lectin domain common to i-CD23, occur sequentially via initial formation of the 37 kDa fragment (Letellier *et al*, *J Exp Med*, 172
[1990] 693-700). An alternative intracellular cleavage pathway leads to a stable 16 kDa
25 fragment differing in the C-terminal domain from i-CD23 (Grenier-Brosette *et al*, *Eur J Immunol*, 22 [1992] 1573-1577).

Several activities have been ascribed to membrane bound i-CD23 in humans, all of which have been shown to play a role in IgE regulation. Particular activities include: a) antigen presentation, b) IgE mediated eosinophil cytotoxicity, c) B cell homing to germinal
30 centres of lymph nodes and spleen, and d) downregulation of IgE synthesis (Delespesse *et al*, *Adv Immunol*, 49, [1991] 149-191). The three higher molecular weight soluble CD23

fragments (Mr 37, 33 and 29 kDa) have multifunctional cytokine properties which appear to play a major role in IgE production. Thus, the excessive formation of s-CD23 has been implicated in the overproduction of IgE, the hallmark of allergic diseases such as extrinsic asthma, rhinitis, allergic conjunctivitis, eczema, atopic dermatitis and anaphylaxis (Sutton and Gould, *Nature*, **366**, [1993] 421-428). Other biological activities attributed to s-CD23 include the stimulation of B cell growth and the induction of the release of mediators from monocytes. Thus, elevated levels of s-CD23 have been observed in the serum of patients having B-chronic lymphocytic leukaemia (Sarfati *et al*, *Blood*, **71** [1988] 94-98) and in the synovial fluids of patients with rheumatoid arthritis (Chomarat *et al*, *Arthritis and Rheumatism*, **36** [1993] 234-242). That there is a role for CD23 in inflammation is suggested by a number of sources. First, sCD23 has been reported to bind to extracellular receptors which when activated are involved in cell-mediated events of inflammation. Thus, sCD23 is reported to directly activate monocyte TNF, IL-1, and IL-6 release (Armant *et al*, vol 180, *J.Exp. Med.*, 1005-1011 (1994)). CD23 has been reported to interact with the B2-integrin adhesion molecules, CD11b and CD11c on monocyte/macrophage (S. Lecoanet-Henchoz *et al*, *Immunity*, vol 3; 119-125 (1995)) which trigger NO₂⁻, hydrogen peroxide and cytokine (IL-1, IL-6, and TNF) release. Finally, IL-4 or IFN induce the expression of CD23 and its release as sCD23 by human monocytes. Ligation of the membrane bound CD23 receptor with IgE/anti-IgE immune complexes or anti CD23 mAb activates cAMP and IL-6 production and thromboxane B2 formation, demonstrating a receptor-mediated role of CD23 in inflammation.

Because of these various properties of CD23, compounds which inhibit the formation of s-CD23 should have twofold actions of a) enhancing negative feedback inhibition of IgE synthesis by maintaining levels of i-CD23 on the surface of B cells, and b) inhibiting the immunostimulatory cytokine activities of higher molecular weight soluble fragments (Mr 37, 33 and 29 kDa) of s-CD23. In addition, inhibition of CD23 cleavage should mitigate sCD23-induced monocyte activation and mediator formation, thereby reducing the inflammatory response.

TNF α is a pro-inflammatory cytokine which is released from stimulated cells by specific cleavage of a 76-amino acid signal sequence in the inactive precursor to generate the mature form. The cleavage of TNF α has been reported to be carried out by a

metalloprotease (Gearing, A.J.H. et al, (1994) Nature 370, 555-557; McGeehan, G.M. et al, (1994) Nature 370, 558-561; Mohler, K.M. et al, (1994) Nature 370, 218-220). Compounds reported to inhibit the cleavage of $\text{TNF}\alpha$ by the TNF processing enzyme can be broadly described as matrix metalloprotease inhibitors, particularly of the hydroxamic acid class.

5 $\text{TNF}\alpha$ is induced in a variety of cell types in response to bacteria, endotoxin, various viruses and parasites, so that one physiological function ascribed to $\text{TNF}\alpha$ is a contribution to the inflammatory response to acute infection by bacteria, parasites, etc (Dinarello, C.A. (1992) Immunol. 4, 133-145). Overproduction of $\text{TNF}\alpha$ has been implicated in disease states such as rheumatoid arthritis, septic shock, Crohn's disease and cachexia (Dinarello, 1992).

10 Inhibition of processing of $\text{TNF}\alpha$ to the mature, active form would therefore be beneficial in the treatment of these inflammatory disorders. $\text{TNF}\alpha$ may also contribute to the destruction of tissue in autoimmune disease although it is not a initiating factor in these diseases.

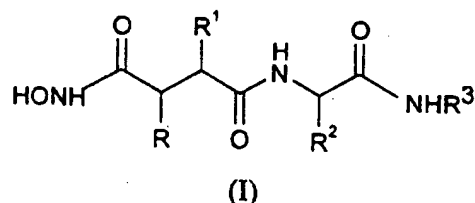
Confirming the importance of $\text{TNF}\alpha$ in rheumatoid arthritis, $\text{TNF}\alpha$ antibodies have been shown to reduce the severity of disease in short term studies in rheumatoid arthritis models

15 (Elliott, M.J., et al (1993) Arthrit. Rheum. 12, 1681-1690; Elliott et al (1994) Lancet 344, 1125-1127).

International Patent Application No. WO 96/02240 (Smithkline Beecham plc) discloses that compounds which inhibit the action of matrix metalloproteases (eg collagenase, stromelysin and gelatinase) are effective inhibitors of the release of human soluble CD23

20 transfected into mammalian cell culture systems.

UK Patent Application No. 9601041.8 (Smithkline Beecham plc) discloses that certain compounds of formula (I) are effective inhibitors of the release of human soluble CD23 transfected into mammalian cell culture systems:



25 According to the present invention, there is provided a compound of formula (I) as defined above wherein:

R is (C_{2-6}) alkenylthiomethyl or (C_{2-6}) alkynylthiomethyl;

R^1 is alkyl or alkenyl;

R² is alkyl, alkenyl, aryl, cycloalkyl or cycloalkenyl; and

R³ is hydrogen, alkyl, alkenyl, alkynyl or aryl.

Suitable values for R include R⁴.CH=CH.CH₂.S.CH₂- and R⁴.C≡C.CH₂.S.CH₂-, wherein R⁴ is (C₁₋₃)alkyl or hydrogen.

5 Alkyl, alkenyl and alkynyl groups referred to herein include straight and branched groups containing up to six carbon atoms and are optionally substituted by one or more groups selected from the group consisting of aryl, heterocyclyl, (C₁₋₆)alkoxy, (C₁₋₆)alkylthio, aryl(C₁₋₆)alkoxy, aryl(C₁₋₆)alkylthio, amino, mono- or di-(C₁₋₆)alkylamino, cycloalkyl, cycloalkenyl, carboxy and esters thereof, hydroxy, and
10 halogen.

Cycloalkyl and cycloalkenyl groups referred to herein include groups having between three and eight ring carbon atoms and are optionally substituted as described hereinabove for alkyl, alkenyl and alkynyl groups.

When used herein, the term "aryl" means single and fused rings suitably containing
15 from 4 to 7, preferably 5 or 6, ring atoms in each ring, which rings, may each be unsubstituted or substituted by, for example, up to three substituents. A fused ring system may include aliphatic rings and need include only one aromatic ring.

Suitable aryl groups include phenyl and naphthyl such as 1-naphthyl or 2-naphthyl.

Suitably any aryl group, including phenyl and naphthyl, may be optionally substituted
20 by up to five, preferably up to three substituents. Suitable substituents include halogen, (C₁₋₆)alkyl, aryl, aryl(C₁₋₆)alkyl, (C₁₋₆)alkoxy, (C₁₋₆)alkoxy(C₁₋₆)alkyl, halo(C₁₋₆)alkyl, aryl(C₁₋₆)alkoxy, hydroxy, nitro, cyano, azido, amino, mono- and di-*N*-(C₁₋₆)alkylamino, acylamino, arylcarbonylamino, acyloxy, carboxy, carboxy salts, carboxy esters, carbamoyl, mono- and di-*N*-(C₁₋₆)alkylcarbamoyl, (C₁₋₆)alkoxycarbonyl, aryloxy carbonyl, ureido,
25 guanidino, sulphonylamino, aminosulphonyl, (C₁₋₆)alkylthio, (C₁₋₆)alkyl sulphinyl, (C₁₋₆)alkylsulphonyl, heterocyclyl and heterocyclyl (C₁₋₆)alkyl. In addition, two adjacent ring carbon atoms may be linked by a (C₃₋₅)alkylene chain, to form a carbocyclic ring.

When used herein the terms "heterocyclyl" and "heterocyclic" suitably include, unless
otherwise defined, aromatic and non-aromatic, single and fused, rings suitably containing up
30 to four heteroatoms in each ring, each of which is selected from oxygen, nitrogen and sulphur, which rings, may be unsubstituted or substituted by, for example, up to three

substituents. Each heterocyclic ring suitably has from 4 to 7, preferably 5 or 6, ring atoms. A fused heterocyclic ring system may include carbocyclic rings and need include only one heterocyclic ring.

Preferably a substituent for a heterocyclyl group is selected from halogen, (C₁-6)alkyl, aryl(C₁-6)alkyl, (C₁-6)alkoxy, (C₁-6)alkoxy(C₁-6)alkyl, halo(C₁-6)alkyl, hydroxy, amino, mono- and di-*N*-(C₁-6)alkyl-amino, acylamino, carboxy salts, carboxy esters, carbamoyl, mono- and di-*N*-(C₁-6)alkylcarbonyl, aryloxy carbonyl, (C₁-6)alkoxycarbonyl(C₁-6)alkyl, aryl, oxy groups, ureido, guanidino, sulphonylamino, aminosulphonyl, (C₁-6)alkylthio, (C₁-6)alkylsulphonyl, (C₁-6)alkylsulphonyl, heterocyclyl and heterocyclyl(C₁-6)alkyl.

In a particular aspect of the invention, R is methyl substituted by propargylthio, 2-butynylthio or allylthio; and/or R¹ is an isobutyl group; and/or R² is a benzyl group; and/or R³ is hydrogen or methyl. In a still further aspect of the invention, each of R to R³ is selected from the group consisting of the values ascribed to it in the Examples hereinbelow.

Preferably, the compound of formula (I) of the invention is selected from the group consisting of the compounds described in the Examples hereinbelow.

According to a further aspect, the present invention provides the use of a compound of formula (I) for the production of a medicament for the treatment or prophylaxis of disorders such as allergy, inflammatory disorders and autoimmune disease in which the overproduction of s-CD23 is implicated.

In a further aspect the invention provides a method for the treatment or prophylaxis of disorders such as allergy, inflammatory disorders and autoimmune disease in which the overproduction of s-CD23 is implicated, which method comprises the administration of a compound of formula (I), to a human or non-human mammal in need thereof.

The invention also provides a pharmaceutical composition for the treatment or prophylaxis of disorders such as allergy, inflammatory disorders and autoimmune disease in which the overproduction of s-CD23 is implicated which comprises a compound of formula (I) and optionally a pharmaceutically acceptable carrier therefor.

According to a further aspect, the present invention provides the use of a compound of formula (I) for the production of a medicament for the treatment or prophylaxis of conditions mediated by TNF, including, but not limited to, inflammation, fever, cardiovascular effects,

haemorrhage, coagulation and acute phase response, cachexia and anorexia, acute infections, shock states, graft versus host reactions and autoimmune disease.

In a further aspect the invention provides a method for the treatment or prophylaxis of conditions mediated by TNF, which method comprises the administration of a compound of formula (I), to a human or non-human mammal in need thereof.

The invention also provides a pharmaceutical composition for the treatment or prophylaxis of conditions mediated by TNF, which comprises a compound of formula (I) and optionally a pharmaceutically acceptable carrier therefor.

Particular inflammatory disorders include CNS disorders such as Alzheimers disease, multiple sclerosis, and multi-infarct dementia, as well as the inflammation mediated sequelae of stroke and head trauma.

It is to be understood that the pharmaceutically acceptable salts, solvates and other pharmaceutically acceptable derivatives of the compound of formula (I) are also included in the present invention.

Salts of compounds of formula (I) include for example acid addition salts derived from inorganic or organic acids, such as hydrochlorides, hydrobromides, hydroiodides, p-toluenesulphonates, phosphates, sulphates, acetates, trifluoroacetates, propionates, citrates, maleates, fumarates, malonates, succinates, lactates, oxalates, tartarates and benzoates.

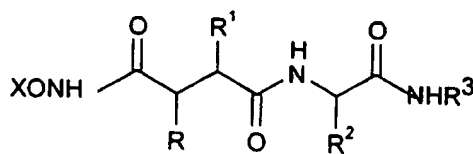
Salts may also be formed with bases. Such salts include salts derived from inorganic or organic bases, for example alkali metal salts such as sodium or potassium salts, and organic amine salts such as morpholine, piperidine, dimethylamine or diethylamine salts.

It has surprisingly been found that the compounds of the present invention are potent inhibitors of CD23 processing and TNF release. Certain compounds of the invention exhibit reduced collagenase inhibitory activity in comparison with the above-mentioned compounds of the prior art.

The compounds of the invention may be prepared by use of any appropriate conventional method, for example by analogy with the methods disclosed in patent publications WO 90/05716, WO 93/24475, WO 94/21625, WO 95/19956, WO 90/05719, WO 91/02716, WO 92/13831, WO 93/20047, EP-A-0214639, EP-A-0236872, EP-A-0274453, EP-A-0489577, EP-A-0489579, EP-A-0497192, EP-A-0574758 and USP 4599361.

Accordingly, a further aspect of the invention provides a process for preparing a compound of formula (I) as defined hereinabove, which process comprises:

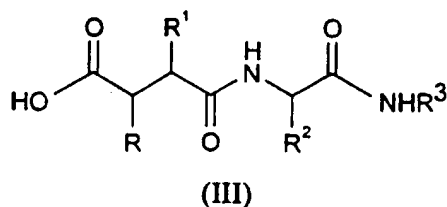
- (a) deprotecting a compound of formula (II):



(II)

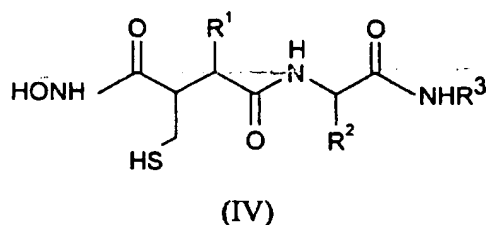
wherein R to R³ are as defined hereinabove, and X is a protecting group such as trimethylsilyl or

(b) reacting a compound of formula (III):



5 wherein R to R³ are as defined hereinabove, with hydroxylamine or a salt thereof, or

(c) reacting a compound of formula (IV):

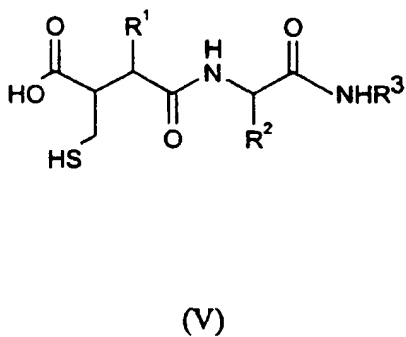


10 wherein R¹ to R³ are as defined hereinabove, with a compound of formula R⁵.X, wherein R⁵ is (C₂₋₆)alkenyl or (C₂₋₆)alkynyl and X is a leaving group such as bromine or iodine; or

(d) converting a compound of formula (I) to a different compound of formula (I) as defined hereinabove.

15 Compounds of formulae (II) and (III) are novel and form a further aspect of the invention.

Compounds of formula (II) can be prepared from compounds of formula (III) by reaction with a protected hydroxylamine. Compounds of formula (III) can be prepared by reacting a compound of formula (V):

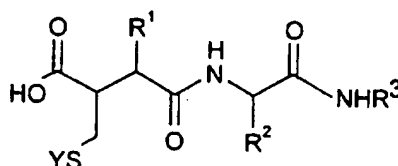


20

wherein R^1 to R^3 are as defined hereinabove, with a compound of formula $R^5.X$ as hereinbefore defined.

Suitable protecting groups for a hydroxamic acid are well known in the art and include trimethylsilyl, t-butyl and t-butyldimethylsilyl.

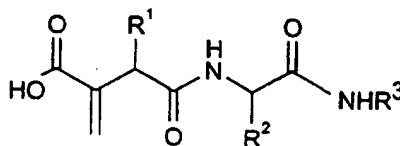
- 5 Compounds of formula (V) can be prepared by hydrolysis of a compound of formula (VI):



(VI)

- 10 wherein R^1 to R^3 are as hereinbefore defined and Y is a protecting group such as alkanoyl or aroyl.

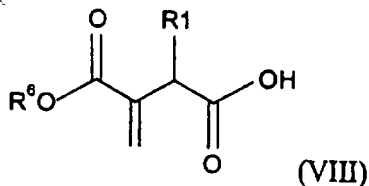
Compounds of formula (VI) can be prepared by reacting a compound of formula (VII):



(VII)

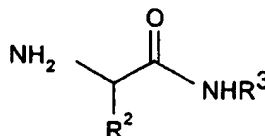
- 15 wherein R^1 to R^3 are as hereinbefore defined, with a compound of formula YSH.

Compounds of formula (VII) can be prepared by reacting a compound of formula (VIII):



(VIII)

- 20 wherein R^1 is as defined hereinabove and R^6 is a protecting group for carboxy such as t-butyl, with a compound of formula (IX):



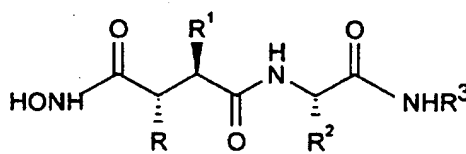
(IX)

wherein R^2 and R^3 are as defined hereinabove, or an activated derivative thereof, and
 5 thereafter removing the carboxy protecting group.

The starting materials and other reagents are available commercially or can be synthesised by well-known and conventional methods. Compounds of formula (VIII) may be prepared as described in WO 94/21612 (Otsuka) at p70.

The isomers, including stereoisomers, of the compounds of the present invention
 10 may be prepared as mixtures of such isomers or as individual isomers. The individual isomers may be prepared by any appropriate method, for example individual stereoisomers may be prepared by stereospecific chemical synthesis starting from chiral substrates or by separating mixtures of diastereoisomers using known methods. In a preferred aspect, the invention provides compounds of formula (IA):

15



(1A)

It is preferred that the compounds are isolated in substantially pure form.

As stated herein an inhibitor of the formation of soluble human CD23 has useful
 20 medical properties. Preferably the active compounds are administered as pharmaceutically acceptable compositions.

The compositions are preferably adapted for oral administration. However, they may be adapted for other modes of administration, for example in the form of a spray, aerosol or other conventional method for inhalation, for treating respiratory tract
 25 disorders; or parenteral administration for patients suffering from heart failure. Other alternative modes of administration include sublingual or transdermal administration.

The compositions may be in the form of tablets, capsules, powders, granules, lozenges, suppositories, reconstitutible powders, or liquid preparations, such as oral or sterile parenteral solutions or suspensions.

5 In order to obtain consistency of administration it is preferred that a composition of the invention is in the form of a unit dose.

Unit dose presentation forms for oral administration may be tablets and capsules and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tableting
10 lubricants, for example magnesium stearate; disintegrants, for example starch, polyvinylpyrrolidone, sodium starch glycollate or microcrystalline cellulose; or pharmaceutically acceptable wetting agents such as sodium lauryl sulphate.

The solid oral compositions may be prepared by conventional methods of blending, filling or tableting. Repeated blending operations may be used to distribute
15 the active agent throughout those compositions employing large quantities of fillers. Such operations are of course conventional in the art. The tablets may be coated according to methods well known in normal pharmaceutical practice, in particular with an enteric coating.

Oral liquid preparations may be in the form of, for example, emulsions, syrups,
20 or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, gelatin, hydroxyethylcellulose, carboxymethylcellulose, aluminium stearate gel, hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan
25 monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as esters of glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid; and if desired conventional flavouring or colouring agents.

30 For parenteral administration, fluid unit dosage forms are prepared utilizing the compound and a sterile vehicle, and, depending on the concentration used, can be either

suspended or dissolved in the vehicle. In preparing solutions the compound can be dissolved in water for injection and filter sterilized before filling into a suitable vial or ampoule and sealing. Advantageously, adjuvants such as a local anaesthetic, a preservative and buffering agents can be dissolved in the vehicle. To enhance the stability, the composition can be frozen after filling into the vial and the water removed under vacuum. Parenteral suspensions are prepared in substantially the same manner, except that the compound is suspended in the vehicle instead of being dissolved, and sterilization cannot be accomplished by filtration. The compound can be sterilized by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the compound.

Compositions of this invention may also suitably be presented for administration to the respiratory tract as a snuff or an aerosol or solution for a nebulizer, or as a microfine powder for insufflation, alone or in combination with an inert carrier such as lactose. In such a case the particles of active compound suitably have diameters of less than 50 microns, preferably less than 10 microns for example diameters in the range of 1-50 microns, 1-10 microns or 1-5 microns. Where appropriate, small amounts of other anti-asthmatics and bronchodilators, for example sympathomimetic amines such as isoprenaline, isoetharine, salbutamol, phenylephrine and ephedrine; xanthine derivatives such as theophylline and aminophylline and corticosteroids such as prednisolone and adrenal stimulants such as ACTH may be included.

The compositions may contain from 0.1% to 99% by weight, preferably from 10-60% by weight, of the active material, depending upon the method of administration. A preferred range for inhaled administration is 10-99%, especially 60-99%, for example 90, 95 or 99%.

Microfine powder formulations may suitably be administered in an aerosol as a metered dose or by means of a suitable breath-activated device.

Suitable metered dose aerosol formulations comprise conventional propellants, cosolvents, such as ethanol, surfactants such as oleyl alcohol, lubricants such as oleyl alcohol, desiccants such as calcium sulphate and density modifiers such as sodium chloride.

Suitable solutions for a nebulizer are isotonic sterilised solutions, optionally buffered, at for example between pH 4-7, containing up to 20mg/ml of compound but more generally 0.1 to 10mg/ml, for use with standard nebulisation equipment.

An effective amount will depend on the relative efficacy of the compounds of the present invention, the severity of the disorder being treated and the weight of the sufferer. Suitably, a unit dose form of a composition of the invention may contain from 0.1 to 1000mg of a compound of the invention (0.001 to 10mg via inhalation) and more usually from 1 to 500mg, for example 1 to 25 or 5 to 500mg. Such compositions may be administered from 1 to 6 times a day, more usually from 2 to 4 times a day, in a manner such that the daily dose is from 1mg to 1g for a 70 kg human adult and more particularly from 5 to 500mg. That is in the range of about 1.4×10^{-2} mg/kg/day to 14 mg/kg/day and more particularly in the range of about 7×10^{-2} mg/kg/day to 7 mg/kg/day.

The following examples illustrate the invention but do not limit it in any way.

BIOLOGICAL TEST METHODS

Procedure 1: The ability of test compounds to inhibit the release of soluble CD23 was investigated by use of the following procedure.

5

RPMI 8866 Cell membrane CD23 cleavage activity assay:

Plasma membranes from RPMI 8866 cells, a human Epstein-Barr virus transformed B-cell line (Sarfati et al., Immunology 60 [1987] 539-547) expressing high levels of CD23 are purified using an aqueous extraction method. Cells resuspended in homogenization
10 buffer (20mM HEPES pH 7.4, 150 mM NaCl, 1.5 mM MgCl₂, 1 mM DTT) are broken by N₂ cavitation in a Parr bomb and the plasma membrane fraction mixed with other membranes is recovered by centrifugation at 10,000Xg. The light pellet is resuspended in 0.2 M potassium phosphate, pH 7.2 using 2 ml per 1-3 g wet cells and the nuclear pellet is
15 discarded. The membranes are further fractionated by partitioning between Dextran 500 (6.4% w/w) and polyethylene glycol (PEG) 5000 (6.4% w/w) (ref), at 0.25 M sucrose in a total of 16 g per 10-15 mg membrane proteins [Morre and Morre, BioTechniques 7, 946-957 (1989)]. The phases are separated by brief centrifugation at 1000Xg and the PEG (upper) phase is collected, diluted 3-5 fold with 20 mM potassium phosphate buffer pH 7.4, and
20 centrifuged at 100,000Xg to recover membranes in that phase. The pellet is resuspended in phosphate-buffered saline and consists of 3-4 fold enriched plasma membranes as well as some other cell membranes (e.g. lysosomes, Golgi). The membranes are aliquoted and stored at -80°C. Fractionation at 6.6 % Dextran/PEG yields plasma membranes enriched 10-fold.

The fractionated membranes are incubated at 37°C for times up to 4 hrs to produce
25 fragments of CD23 which are separated from the membrane by filtration in 0.2 micron Durapore filter plates (Millipore) after quenching the assay with 5 uM Preparation 1 from P 30994. sCD23 released from the membrane is determined using the EIA kit from The Binding Site (Birmingham, UK) or a similar one utilizing MHM6 anti-CD23 mAb [Rowe et al., Int. J. Cancer, 29, 373-382 (1982)] or another anti-CD23 mAb as the capture antibody in a
30 sandwich EIA.. The amount of soluble CD23 made by 0.5 ug membrane protein in a total volume of 50 ul phosphate-buffered saline is measured by EIA and compared to the amount

made in the presence of various concentrations of inhibitors. Inhibitors are prepared in solutions of water or dimethylsulfoxide (DMSO) and the final DMSO concentration is not more than 2 %. IC₅₀'s are determined by curve fitting as the concentration where 50 % inhibition of production of sCD23 is observed relative to the difference in sCD23 between controls incubated without inhibitor.

Procedure 2: The ability of test compounds to inhibit collagenase was investigated using the following procedure.

10 Collagenase inhibition assay:

The potency of compounds to act as inhibitors of collagenase was determined by the method of Cawston and Barrett (Anal. Biochem. 99, 340-345, 1979), hereby incorporated by reference, whereby a 1 mM solution of the inhibitor being tested or dilutions thereof, was incubated at 37 °C for 18 h with collagen and human recombinant collagenase, from synovial fibroblasts cloned, expressed and purified from E. Coli, (buffered with 150 mM Tris, pH 7.6, containing 15 mM calcium chloride, 0.05% Brij 35, 200 mM sodium chloride and 0.02% sodium azide). The collagen was acetylated ³H type 1 bovine collagen prepared by the method of Cawston and Murphy (methods in Enzymology 80, 711, 1981). The samples were centrifuged to sediment undigested collagen and an aliquot of the radioactive supernatant removed for assay on a scintillation counter as a measure of hydrolysis. The collagenase activity in the presence of 1mM inhibitor, or dilution thereof, was compared to activity in a control devoid of inhibitor and the results reported as that concentration effecting 50% of the collagenase (IC₅₀).

Procedure 3: The ability of test compounds to inhibit TNF release was investigated using the following procedure.

Assay for inhibition of release of TNF α from human monocytes stimulated by lipopolysaccharide (LPS) endotoxin.

Human monocytes, cultured in RPMI 1640 medium supplemented with 10 % fetal calf serum, are centrifuged at 1000Xg for 5 min and then resuspended in medium at 2×10^6 cells/ml. The cell suspension is aliquoted in 24 well plates, 1 ml per well. Compounds to be tested are dissolved in neat dimethyl sulfoxide (DMSO) and added to culture with the final

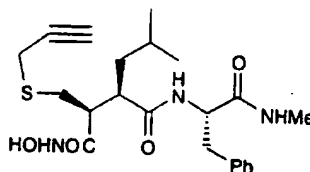
5 DMSO concentration at 0.1 %. Compounds are added to cells in triplicate wells. TNF α release is stimulated by addition of LPS to the cells at a final concentration of 200 ng/ml. Appropriate control cultures are set up in triplicates also. The plates are incubated for 18-20 hrs at 37° C, 5% CO₂, then centrifuged at 1000 Xg for 5 min. A specific ELISA for human TNF α (SmithKline Beecham) is used to measure TNF levels in the cell-free culture

10 supernatants.

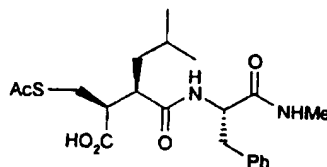
Example 1

N-[(4-Hydroxyamino-2-(R)-isobutyl-3-(S)-propargylthiomethyl)succinyl]-(S)-phenylalanine-N'-methylamide

5



a) N-[(3-(S)-Acetylthiomethyl-2-(R)-isobutyl)succinyl]-(S)-phenylalanine-N'-methylamide



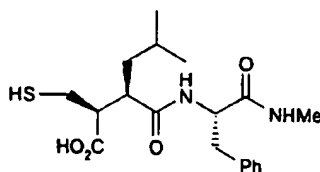
10

A solution of N-(2-(R)-isobutyl-3-methylenesuccinyl)-(S)-phenylalanine-N'-methylamide (1g, 2.9mmol) in thiolacetic acid (6ml) was stirred for 16h at 20°C. The excess thiol was evaporated and co-evaporated with toluene (x2). The resultant solid was triturated with ether to give the acetylthio compound as a white solid (990mg, 81%)

15

δ_H [(CD₃)₂SO] 0.77 (6H, dd, J=6.6,16.2Hz), 0.89 (1H,m), 1.27 (1H,m), 1.48 (1H,m), 2.25 (3H,s), 2.29-2.51 (4H,m), 2.56 (3H, d, J=4.4Hz), 2.80 (1H, dd, J=9.9,13.5Hz), 2.93 (1H, dd, J=8.8,13.7Hz), 4.54 (1H,m), 7.23 (5H,m), 7.84 (1H,m), 8.35 (1H, d, J=8.5Hz).

b) N-[(2-(R)-Isobutyl-3-(S)-mercaptomethyl)succinyl]-(S)-phenylalanine-N'-methylamide

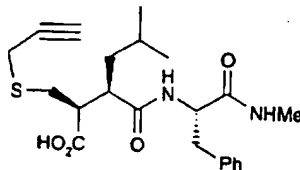


- 5 A solution of N-[(3-(S)-acetylthiomethyl-2-(R)-isobutyl)succinyl]-(S)-phenylalanine-N'-methylamide (850mg, 2mmol) in methanol (50ml) was treated with 1M sodium hydroxide solution (4ml) and stirred during the passage of argon gas through the solution for 2h. The solution was acidified with 2M hydrochloric acid and evaporated to a white solid. Trituration with water and ether gave the thiol as a white solid (690mg, 90%)

10

δ H [(CD₃)₂SO] 0.73 (3H, d, J=6.6Hz), 0.79 (3H, d, J=6.3Hz), 0.88 (1H,m), 1.26 (1H,m), 1.45 (1H,m), 1.93 (1H, t, J=7.5Hz), 2.09 (1H,m), 2.36 (3H,m), 2.58 (3H, d, J=4.7Hz), 2.77 (1H,m), 2.92 (1H,m), 4.54 (1H,m), 7.26 (5H,m), 7.86 (1H,m), 8.30 (1H, d, J=8.5Hz), 12.40 (1H,br.s).

c) N-[(2-(R)-Isobutyl-3-(S)-propargylthiomethyl)succinyl]-(S)-phenylalanine-N'-methylamide

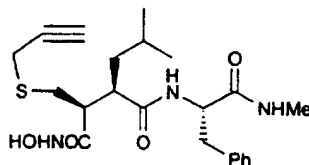


5

To a solution of N-[(2-(R)-isobutyl-3-(S)-mercaptomethyl)succinyl]-(S)-phenylalanine-N'-methylamide (440mg, 1.04mmol) in methanol (20ml) was added 1M sodium hydroxide (2.1ml, 2.1mmol) and propargyl bromide (0.17ml, 1.14mmol) at 20°C under argon and stirred for 3h. The solution was acidified with 2M hydrochloric acid and evaporated. The residue was triturated with ether to give the acid as white flakes (230mg, 75%)

10
15
 δ H [(CD₃)₂SO] 0.74 (3H, d, J=6.6Hz), 0.80 (3H, d, J=6.5Hz), 0.90 (1H, ddd, J=2.8,10.1,13.4Hz), 1.30 (1H,m), 1.48 (1H, ddd, J=3.6,10.6,13.2 Hz), 2.05 (1H, dd, J=2.2, 12.3Hz), 2.38 (1H,m), 2.43 (2H,m), 2.57 (3H, d, J=4.6Hz), 2.79 (1H, dd, J=10.1,13.7Hz), 2.94 (1H, dd, J=5.0,13.7Hz), 3.06 (1H,m), 3.09 (1H,m), 3.15 (1H,m), 4.53 (1H, ddd, J=5.0,8.6,10.0 Hz), 7.18 (1H,m), 7.27 (4H,m), 7.82 (1H, q, J=4.6Hz), 8.28 (1H, d, J=8.5Hz), 12.42 (1H,br.s).

d) N-[4-Hydroxyamino-2-(R)-isobutyl-3-(S)-propargylthiomethyl)succinyl]-(S)-phenylalanine-N'-methanamide



5

A solution of N-[(2-(R)-isobutyl-3-(S)-propargylthiomethyl)succinyl]-(S)-phenylalanine-N'-methanamide (105mg, 0.25mmol) in N,N-dimethylformamide (5ml) was treated with 1-hydroxy-7-azabenzotriazole (44mg, 0.33mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (63mg, 0.33mmol) and stirred at 20°C for 2h under argon.

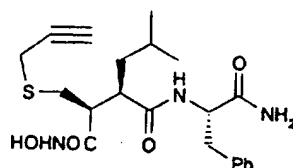
- 10 Treatment of the solution with N-methylmorpholine (31ul, 0.28mmol) and hydroxylamine hydrochloride (20mg, 0.28mmol) was followed by stirring of the reaction for 16h at 20°C. The reaction was evaporated and the residue partitioned between ethyl acetate and sodium hydrogen carbonate solution. The organic layer was successively washed with 10% citric acid and brine and dried (MgSO₄). The residue from evaporation was triturated with ether to
- 15 give the hydroxamic acid as a white solid (30mg, 28%). m.p. 222-224°C.

- δ H [(CD₃)₂SO] 0.74 (3H, d, J=6.5Hz), 0.80 (3H, d, J=6.5Hz), 0.88 (1H, ddd, J=3.1,10.0,12.9Hz), 1.32 (1H,m), 1.37 (1H,m), 1.85 (1H, dd, J=3.0, 13.2Hz), 2.13 (1H, ddd, J=3.0,11.0,11.0Hz), 2.35 (1H, dd, J=11.7,13.1Hz), 2.41 (1H, ddd, J=3.0,10.8,10.8Hz), 2.57
- 20 (3H, d, J=4.6Hz), 2.79 (1H, dd, J=10.2,13.7Hz), 2.94 (1H, dd, J=4.9,13.7Hz), 2.96 (1H, dd, J=2.7,16.3Hz), 3.03 (1H, t, J=2.6Hz), 3.12 (1H, dd, J=2.7,16.3Hz), 4.53 (1H, ddd, J=4.9,8.6,10.1Hz), 7.18 (1H,m), 7.27 (4H,m), 7.77 (1H, q, J=4.6Hz), 8.25 (1H, d, J=8.5Hz), 8.85 (1H, d, J=1.7Hz), 10.49 (1H, d, J=1.5Hz).

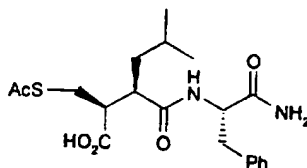
Example 2

N-[(4-Hydroxyamino-2-(R)-isobutyl-3-(S)-propargylthiomethyl)succinyl]-(S)-phenylalaninamide

5



a) N-[(3-(S)-Acetylthiomethyl-2-(R)-isobutyl)succinyl]-(S)-phenylalaninamide

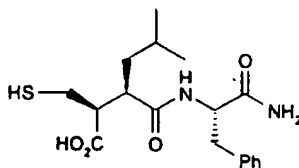


10

Prepared as in Example 1a) using N-(2-(R)-isobutyl-3-methylenesuccinyl)-(S)-phenylalaninamide and the title compound isolated in 53% yield.

15 δ H [(CD₃)₂SO] 0.75 (3H, d, J=6.1Hz), 0.81 (3H, d, J=6.1Hz), 0.89 (1H,m), 1.31 (1H,m), 1.49 (1H,m), 2.30-2.56 (7H,m), 2.79 (1H, t, J=12Hz), 2.99 (1H,m), 4.57 (1H,m), 7.00 (1H,s), 7.22 (5H,m), 7.36 (1H,s), 8.29 (1H, d, J=8.5Hz), 12.47 (1H,br.s).

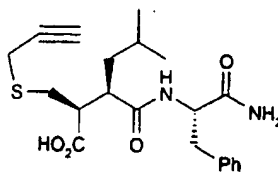
b) N-[(2-(R)-Isobutyl-3-(S)-mercaptomethyl)succinyl]-(S)-phenylalaninamide



5 Prepared as in Example 1b) using N-[(3-(S)-acetylthiomethyl-2-(R)-isobutyl)succinyl]-(S)-phenylalaninamide and the title compound isolated in 72% yield.

δ H [(CD₃)₂SO] 0.62 (1H,m), 0.70 (3H, d, J=6.6Hz), 0.76 (3H, d, J=6.3Hz), 0.98 (1H, t, J=10.0Hz), 1.23 (1H,m), 1.42 (1H, t, J=12.1Hz), 1.70 (1H, d, J=9.3Hz), 2.27 (3H,m), 2.76
10 (1H,m), 3.00 (1H, dd, J=4.1,13.5 Hz), 4.48 (1H,m), 6.99 (1H,s), 7.27 (5H,m), 7.47 (1H,s), 8.52 (1H, d, J=8.5Hz).

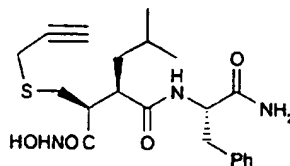
c) N-[(2-(R)-Isobutyl-3-(S)-propargylthiomethyl)succinyl]-(S)-phenylalaninamide



- 5 Prepared as in Example 1c) using N-[(2-(R)-isobutyl-3-(S)-mercaptomethyl)succinyl]-(S)-phenylalaninamide and the title compound isolated in 45% yield.

10 δ H [(CD₃)₂SO] 0.74 (3H, d, J=6.3Hz), 0.80 (3H, d, J=6.3Hz), 0.89 (1H, t, J=10.7Hz), 1.30 (1H,m), 1.48 (1H,m), 2.02 (1H, d, J=9.6Hz), 2.41 (3H,m), 2.79 (1H,m), 2.98 (1H,m), 3.09 (3H,m), 4.55 (1H,m), 7.01 (1H,s), 7.27 (5H,m), 7.35 (1H,s), 8.23 (1H, d, J=8.8Hz), 12.44 (1H,br.s).

d) N-[4-Hydroxyamino-2-(R)-isobutyl-3-(S)-propargylthiomethyl)succinyl]-(S)-phenylalaninamide



5

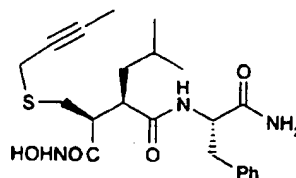
Prepared as in Example 1d) using N-[(2-(R)-isobutyl-3-(S)-propargylthiomethyl)succinyl]-(S)-phenylalaninamide and the title compound isolated in 77% yield. m.p. 228-231°C.

10 δ H [(CD₃)₂SO] 0.75 (3H, d, J=6.5Hz), 0.81 (3H, d, J=6.5Hz), 0.87 (1H,m), 1.35 (2H,m), 1.82 (1H, dd, J=3.0, 13.2Hz), 2.12 (1H,m), 2.31 (1H, d, J=13.0Hz), 2.40 (1H,m), 2.78 (1H, dd, J=10.5,13.7Hz), 2.92 (1H, d, J=2.7Hz), 2.97 (1H, d, J=2.7Hz), 3.03 (1H, t, J=2.7Hz), 3.10 (1H, dd, J=2.7,16.2Hz), 4.53 (1H,m), 6.98 (1H,s), 7.29 (6H,m), 8.20 (1H, d, J=8.6Hz), 8.84 (1H,s), 10.49 (1H,s).

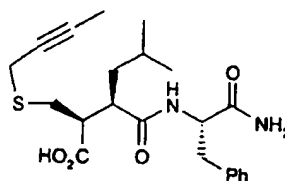
Example 3

N-[(3-(S)-(2-Butynylthiomethyl)-4-hydroxyamino-2-(R)-isobutyl)succinyl]-(S)-phenylalaninamide

5



a) N-[(3-(S)-(2-butynylthiomethyl)-2-(R)-isobutyl)succinyl]-(S)-phenylalaninamide

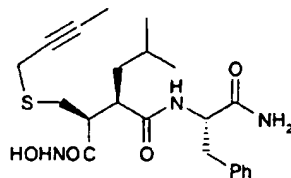


10

Prepared as in Example 2c) and the title compound isolated in 40% yield.

15 δ H [(CD₃)₂SO] 0.74 (3H, d, J=6.3Hz), 0.80 (3H, d, J=6.3Hz), 0.89 (1H, t, J=10.7Hz), 1.31 (1H,m), 1.49 (1H,m), 1.75 (3H,m), 2.03 (1H,m), 2.25 (1H,m), 2.41 (2H,m), 2.78 (1H, dd, J=10.3,13.7Hz), 2.99 (1H, dd, J=4.6,14.5Hz), 3.06 (2H, dd, J=2.6,6.6Hz), 4.55 (1H,m), 6.98 (1H,s), 7.28 (6H,m), 8.23 (1H, d, J=8.6Hz), 12.38 (1H,br.s).

b) N-[(3-(S)-(2-Butynylthiomethyl)-4-hydroxyamino-2-(R)-isobutyl)succinyl]-(S)-phenylalaninamide



5

Prepared as in Example 2d) and the title compound isolated in 75% yield. m.p. 209-212°C.

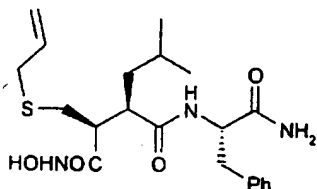
δ H [(CD₃)₂SO] 0.74 (3H, d, J=6.3Hz), 0.80 (3H, d, J=6.2Hz), 0.86 (1H, t, J=11.0Hz), 1.38 (2H, m), 1.74 (4H, m), 2.15 (1H, m), 2.29 (1H, m), 2.40 (1H, m), 2.77 (1H, m), 2.95 (3H, m), 4.54 (1H, m), 6.99 (1H, s), 7.28 (6H, m), 8.22 (1H, d, J=8.5Hz), 8.84 (1H, s), 10.49 (1H, s)

10

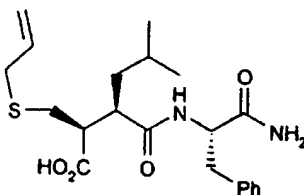
Example 4

N-[(3-(S)-(allylthiomethyl)-4-hydroxyamino-2-(R)-isobutyl)succinyl]-(S)-phenylalaninamide

5



a) N-[(3-(S)-(allylthiomethyl)-2-(R)-isobutyl)succinyl]-(S)-phenylalaninamide

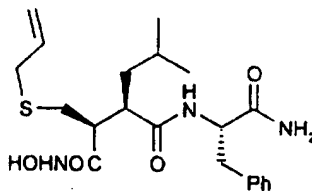


10

Prepared as in Example 2c) and the title compound isolated in 29% yield.

δ H [(CD₃)₂SO] 0.74 (3H, d, J=6.5Hz), 0.79 (3H, d, J=6.5Hz), 0.90 (1H, t, J=10.4Hz), 1.30
 15 (1H,m), 1.46 (1H,m), 1.86 (1H,d, J = 11.8Hz), 2.28 (1H,m), 2.37 (2H,d, J = 9.9Hz), 2.77
 (1H,dd, J = 10.5, 13.7Hz), 2.91 (2H, m), 2.96 (1H,dd, J=4.4,13.7Hz), 4.51 (1H,m), 4.95 (2H,
 m), 5.59 (1H, m), 6.97 (1H,s), 7.27 (5H,m), 7.36 (1H, s), 8.29 (1H, br s), 12.35 (1H,br.s).

b) N-[(3-(S)-(allylthiomethyl)-4-hydroxyamino-2-(R)-isobutyl)succinyl]-(S)-phenylalaninamide



5

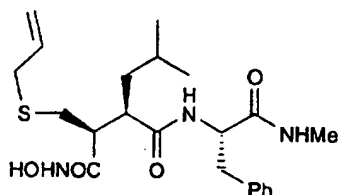
Prepared as in Example 2d) and the title compound isolated in 86% yield. m.p. 230-232°C.

δ_{H} [(CD₃)₂SO] 0.75 (3H, d, J=6.5Hz), 0.81 (3H, d, J=6.5Hz), 0.88 (1H, t, J=10.0Hz), 1.37
 (2H,m), 1.68 (1H,dd, J = 2.0,12.6Hz), 2.16 (2H,m), 2.36 (1H,m), 2.82 (3H,m), 2.96 (1H, dd, J
 10 = 4.3, 13.7Hz), 4.50 (1H,m), 4.92 (2H, m), 5.55 (1H, m), 6.97 (1H,s), 7.28 (6H,m), 8.18 (1H,
 d, J = 8.6Hz), 8.80 (1H, d, J = 1.9Hz), 10.48 (1H, d, J = 1.9Hz).

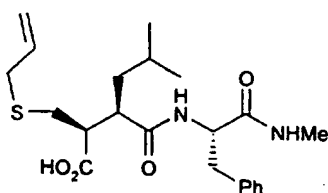
Example 5

N-[(3-(S)-(allylthiomethyl)-4-hydroxyamino-2-(R)-isobutyl)succinyl]-(S)-phenyl N'-methylamide

5



a) N-[(3-(S)-(allylthiomethyl)-2-(R)-isobutyl)succinyl]-(S)-phenyl N'-methylamide

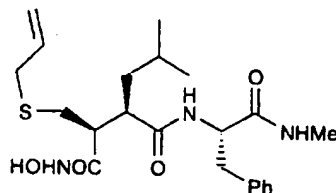


10

Prepared as in Example 1c) and the title compound isolated in 66% yield.

15 δ H [(CD₃)₂SO] 0.74 (3H, d, J=6.4Hz), 0.80 (3H, d, J=6.4Hz), 0.87 (1H, t, J=10.7Hz), 1.28 (1H,m), 1.47 (1H,m), 1.84 (1H,d, J = 11.6Hz), 2.19 (1H,m), 2.24 (2H,m), 2.57 (3H, d, J = 4.6Hz), 2.77 (1H,m), 2.90 (3H, m), 4.51 (1H,m), 4.95 (2H, m), 5.58 (1H, m), 7.26 (5H,m), 7.85 (1H,m), 8.30 (1H, d, J = 9.0 Hz)), 12.35 (1H,br.s).

b) N-[(3-(S)-(allylthiomethyl)-4-hydroxyamino-2-(R)-isobutyl)succinyl]-(S)-phenyl-N-methylamide



5

Prepared as in Example 2d) and the title compound isolated in 81% yield. m.p. 228-230°C.

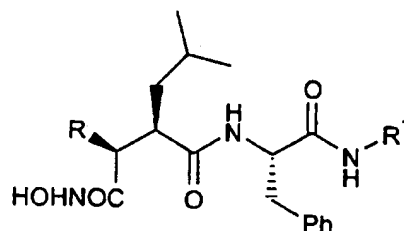
$\delta_{\text{H}}[(\text{CD}_3)_2\text{SO}]$ 0.74 (3H, d, $J=6.3\text{Hz}$), 0.80 (3H, d, $J=6.3\text{Hz}$), 0.86 (1H, t, $J=10.0\text{Hz}$), 1.35
 (2H, m), 1.69 (1H, d, $J = 11.3\text{Hz}$), 2.15 (2H, m), 2.40 (1H, m), 2.56 (3H, d, $J = 4.4\text{Hz}$), 2.84
 10 (4H, m), 4.49 (1H, m), 4.90 (2H, m), 5.56 (1H, m), 7.26 (5H, m), 7.78 (1H, m), 8.25 (1H, d, $J =$
 8.6Hz), 8.82 (1H, d, $J = 1.7\text{Hz}$), 10.49 (1H, d, $J = 1.7\text{Hz}$).

Activity Data

5

Compound	CD23 proteinase inhibition % at 1uM	Collagenase inhibition IC50 uM	TNF processing inhibition % at 1uM
Example 1	91	>10	83.0±2.5
Comparative Example*	96	0.005	76.4±0.9

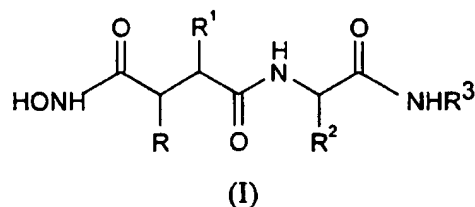
*The comparative example was Example 2 of WO 90/05719, the compound of formula:



10 wherein R is CH₂S-(2-thienyl) and R¹ is methyl.

Claims

1. A compound of formula (I):



wherein:

R is (C₂₋₆)alkenylthiomethyl or (C₂₋₆)alkynylthiomethyl;

R¹ is alkyl or alkenyl;

R² is alkyl, alkenyl, aryl, cycloalkyl or cycloalkenyl; and

10 R³ is hydrogen, alkyl, alkenyl, alkynyl or aryl.

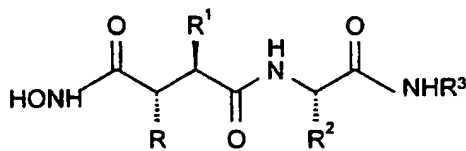
2. A compound according to claim 1, wherein R is R⁴.CH=CH.CH₂.S.CH₂- or R⁴.C≡C.CH₂.S.CH₂-, wherein R⁴ is (C₁₋₃)alkyl or hydrogen.

- 15 3. A compound according to claim 2, wherein R is methyl substituted by propargylthio, 2-butenylthio or allylthio; and/or R¹ is an isobutyl group; and/or R² is a benzyl group; and/or R³ is hydrogen or methyl.

4. A compound according to claim 2, wherein each of R to R³ is selected from the group
20 consisting of the values ascribed to it in the Examples hereinabove.

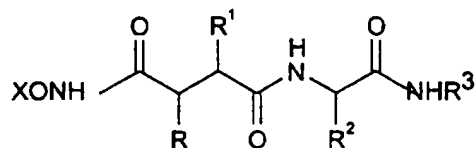
5. A compound according to claim 2, selected from the group consisting of the compounds described in the Examples hereinabove.

- 25 6. A compound according to claim 1 or 2, which is a compound of formula (IA):



(1A)

7. The use of a compound according to any one of the preceding claims for the production of a medicament for the treatment or prophylaxis of disorders such as allergy,
5 inflammatory disorders and autoimmune disease in which the overproduction of s-CD23 is implicated.
8. A method for the treatment or prophylaxis of disorders such as allergy, inflammatory disorders and autoimmune disease in which the overproduction of s-CD23 is implicated,
10 which method comprises the administration of a compound according to any one of claims 1 to 6 to a human or non-human mammal in need thereof.
9. A pharmaceutical composition for the treatment or prophylaxis of disorders such as allergy, inflammatory disorders and autoimmune disease in which the overproduction of s-
15 CD23 is implicated which comprises a compound according to any one of claims 1 to 6 and optionally a pharmaceutically acceptable carrier therefor.
10. The use of a compound according to any one of claims 1 to 6 for the production of a medicament for the treatment or prophylaxis of conditions mediated by TNF, including, but
20 not limited to, inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia and anorexia, acute infections, shock states, graft versus host reactions and autoimmune disease.
11. A method for the treatment or prophylaxis of conditions mediated by TNF, which
25 method comprises the administration of a compound according to any one of claims 1 to 6 to a human or non-human mammal in need thereof.
12. A process for preparing a compound according to any one of claims 1 to 6, which process comprises:
30 (a) deprotecting a compound of formula (II):

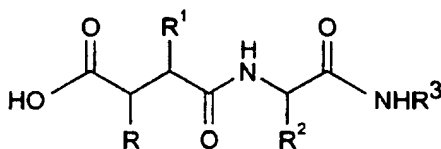


(II)

wherein R to R³ are as defined hereinabove, and X is a protecting group; or

(b) reacting a compound of formula (III):

5

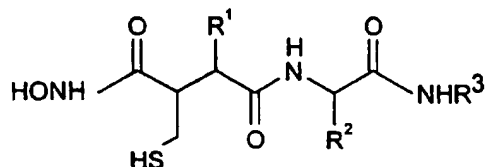


(III)

wherein R to R³ are as defined hereinabove, with hydroxylamine or a salt thereof; or

(c) reacting a compound of formula (IV):

10



(IV)

wherein R¹ to R³ are as defined hereinabove, with a compound of formula R⁵.Y, wherein R⁵ is (C₂₋₆)alkenyl or (C₂₋₆)alkynyl and Y is a leaving group; or

15 (d) converting a compound of formula (I) to a different compound of formula (I) as defined hereinabove.

13. A compound of formula (II) as defined in claim 12.

20 14. A compound of formula (III) as defined in claim 12.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 97/02500

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C07C259/06 A61K31/185

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 97 26257 A (SMITHKLINE BEECHAM PLC) 24 July 1997 cited in the application see page 5, line 1-10 ---	1-9, 12-14
A	WO 96 02240 A (SMITHKLINE BEECHAM PLC) 1 February 1996 cited in the application see the whole document ---	1-14
A	WO 90 05719 A (BRITISH BIO-TECHNOLOGY LIMITED) 31 May 1990 cited in the application see the whole document -----	1-14

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

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- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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- *&* document member of the same patent family

Date of the actual completion of the international search

19 August 1997

Date of mailing of the international search report

04.09.97

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 97/02500

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